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## Request for grant of a patent

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| | | |
|---|---|---|
| 1. | Your reference | PCB/P089100PGB |
| 2. | Patent application number *(The Patent Office will fill in this part)* | 0208516.5<br>15 APR 2002 |
| 3. | Full name, address and postcode of the or of each applicant *(underline all surnames)* | THE UNIVERSITY OF LIVERPOOL<br>SENATE HOUSE<br>ABERCROMBY STREET<br>LIVERPOOL<br>L69 3BX |
| | Patents ADP number *(if you know it)* | 7736630001 |
| | If the applicant is a corporate body, give the country/state of its incorporation | UNITED KINGDOM |
| 4. | Title of the invention | CHEMOTHERAPY |
| 5. | Name of your agent *(if you have one)* | Marks & Clerk |
| | "Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)* | 43 Park Place<br>Leeds<br>LS1 2RY |
| | Patents ADP number *(if you know it)* | 18013 |
| 6. | If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and *(if you know it)* the or each application number | Country / Priority application number *(if you know it)* / Date of filing *(day/month/year)* |
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| 8. | Is a statement of inventorship and of right to grant of a patent required in support of this request? *(Answer 'Yes' if:*<br>*a) any applicant named in part 3 is not an inventor, or*<br>*b) there is an inventor who is not named as an applicant, or*<br>*c) any named applicant is a corporate body.*<br>*See note (d))* | YES |



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DUPLICATE



# CHEMOTHERAPY

The present invention relates to the treatment of medical conditions using a combination of chemotherapeutic agents.

In general, when chemotherapy is used for the treatment of human cancers and the like, a combination of agents is employed. In the past, the reasoning behind the choice of which particular combinations of agents are used has been essentially a pragmatic decision, often based more on tolerances to toxicity rather than specific targets.

Recent studies of the process of carcinogenesis, have revealed that many of the genetic lesions involved, cause errors in the cell division/death pathways. The molecular changes that result from such lesions initiate the cancer process. Due to this the molecules involved in such changes provide potentially highly specific targets for chemotherapy. Using the targets identified by this approach new therapeutic agents may be introduced into the clinic. However, to achieve optimal clinical benefit from these agents, they may too need to be used in combination with other anticancer drugs. Again the choice of which particular combinations of agents are used has been a decision based more on tolerances to toxicity rather than specific targets.

According to a first aspect of the present invention, there is provided a use of a first agent that attenuates Topoisomerase I activity and a second agent that inhibits Heat Shock Protein 90 activity for the manufacture of a medicament for contemporaneous or sequential administration in chemotherapy.

According to a second aspect of the present invention, there is provided a method for conducting chemotherapy comprising contemporaneously or sequentially administering to a person or animal in need of said treatment a therapeutically effective amount of a first agent that attenuates Topoisomerase I activity and a second agent that inhibits Heat Shock Protein 90 activity.

DNA. A new enzyme-DNA phosphodiester bond is formed in the process. The covalent DNA-enzyme intermediate can be readily attacked by the free end of the DNA because the enzyme-DNA phosphodiester bond is of comparable energy to the orginal bond.

Sequences for Topoisomerase I are known to the art. Examples of sequences for known Topo I enzymes may be found in the following papers/gene databases:

(a) Human Topo I
D'Arpa *et al.* (1988) Proc Natl Acad Sci U S A 85(8):2543-7; NCBI pubmed nucleotide LOCUS HUMTOPI, ACCESSION J03250

(b) Yeast Topo I
Thrash *et al* (1985) Proc Natl Acad Sci U S A 82(13):4374-8; NCBI pubmed nucleotide LOCUS YSCTOPI, ACCESSION K03077

(c) E.coli Topo I
Tse-Dinh *et al.* (1986) J Mol Biol 191(3):321-31; NCBI pubmed nucleotide LOCUS ECTOPA, ACCESSION X04475 X12873

Human Topo I is of considerable biomedical importance because it is the main target of camptothecin (CPT) family of anticancer drugs. These drugs act by prolonging the lifetime of the nicked intermediate in the Topo I reaction which are presumed to form obstacles to the advancement of transcription and replication complexes that eventually lead to DNA damage and cell death.

The inventor has found that the combined use of a first agent that attenuates Topoisomerase I activity and a second agent that inhibits Heat Shock Protein 90 activity is highly effective for effecting chemotherapy. The first and second agents may be administered contemporaneously (e.g. as a composition according to the third aspect of the invention) or sequentially. If administered sequentially the first and second agents should be therapeutically active within the subject being treated at the same time.

Chemotherapy with first and second agents according to the invention is particularly useful because such therapy results in surprising synergistic actions. The inventors have found that disruption of the interaction between Topo I and HSP90 causes an increase in DNA damage and thereby kills 3 - 5 time (or more) the number of proliferating cells in comparison with what is achievable with a monotherapy. Furthermore satisfactory therapy may be effected using lower doses than would be required in a monotherapy. This has the advantage that the toxic side-effects associated with high doses of chemotherapeutic agents may be obviated or reduced. For instance, damage to health tissues (and other associated side effects of high dose chemotherapy - e.g. sickness, hair loss) may be reduced in human cancer chemotherapy by using lower doses of the combined agents according to the invention (than would be required in a monotherapy) without comprising the efficacy of the treatment.

The invention is based upon studies that have been orientated towards the rational design of chemotherapeutic regimens. The inventor realised that drug development up to the present time has only been directed against single molecule targets and that rational selection of combination chemotherapy may be based on investigating the mechanisms of action of chemotherapeutic agents and identifying potential interaction at the cellular targets of such agents. The inventor's studies established that Topo I and HSP90 interact and lead to the realisation that a combination of agents that specifically inhibit the individual proteins will have great efficacy in chemotherapy. Further experimentation (see the Example) established that treatment of cells with a combination of agents according to the invention was highly

(i) compounds that bind to Topo I and inhibit its activity (e.g. competitive inhibitors; allosteric inhibitors, cleavable complex inhibitors etc);
(ii) compounds which prevent the transcription, translation or expression of Topo I (e.g. ribozymes or antisense DNA molecules e.g. antisense crossing the first intron/exon boundary);
(iii) compounds which inhibit release of Topo I from intracellular stores; and
(iv) compounds which increase the rate of degradation of Topo I.

Examples of compounds that may be used as first agents are well known to the art. For instance, Pommier *et al.* (Biochimica et Biophysica Acta (1998) 1400 p83 – 106) disclose drugs targeted to Topo I as well as mechanism of action for Topo I. Such drugs are incorporated herein by reference as examples of preferred first agents.

The compound may be a Topo I poison or a Topo I suppressor – e.g. as disclosed in Table 1 Pommier *et al.* (*supra*).

Preferred compounds may attenuate the activity of human Topo I.

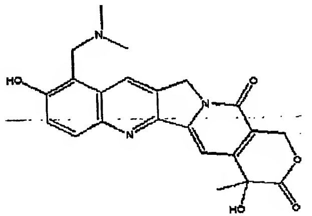
It is preferred that the first agent is Camptothecin (NSC 94600; and CAS Registry Number: 7689034) or a derivative thereof. Examples of preferred derivatives of Camptothecin are disclosed in Pommier *et al.* (*supra*) e.g. see Fig 3 of the paper.

Camptothecin has the following names and structure:

Camptothecin
Camptothecine (8CI)
CAMPTOTHECIN
NSC 100880
1H-Pyrano[3',4';6,7]indolizino[1,2-b]quinoline-3,14(4H,12H)-dione, 4-ethyl-4-hydroxy-, (S)- (9CI)
20(S)-Camptothecine
21, 22-Secocamptothecin-21-oic acid lactone

A further preferred first agent is Camptosar (CPT-11_ or a derivative thereof.

Camptosar (CPT-11)




Several classes of compound may be used according to the invention as the second agent. These compounds include:

(i) compounds that bind to HSP90 and inhibit its activity (e.g. competitive inhibitors or allosteric inhibitors);

(ii) compounds which prevent the transcription, translation or expression of HSP90 (e.g. ribozymes or antisense DNA molecules);

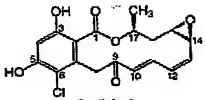
(iii) compounds which inhibit release of HSP90 from intracellular stores; and

(iv) compounds which increase the rate of degradation of HSP90.

Geldanamycin and its derivatives (e.g. 17-Allylamino,17-demethoxygeldanamycin – 17-AAG or Macbecin II) are preferred second agents for use according to the present invention.

**Macbecin II**
Geldanamycin, 18,21-didehydro-6,17-didemethoxy-18,21-dideoxo-18, 21-dihydroxy-15-methoxy-6-methyl-11-O-methyl-, (6S,15R)- (9CI)

**Radicicol**



Radicicol

CNF-101 is a semi-synthetic derivative of Geldanamycin from Conforma Therapeutics (see www.conformacorp.com) and is a further preferred second agent.

It will be appreciated that agents may be developed that have a dual action in that they are able to attenuate Topo I activity and also inhibit Hsp90. Such agents may be used in an adaption of the present invention which involves the use of a single, dual action, agent only rather than separate first and second agents.

The first and second agents may be further combined with other therapeutics when there is a medical need. For instance, for certain medical conditions, the inventor has found even greater therapeutic efficacy when the agents are combined with a mediacment which suppresses apoptosis in non-cancerous tissue (eg pifithrin-α).

Agents which attenuate Topo I activity and inhibit Hsp90 may be used in chemotherapy to treat a number of conditions requiring the induction of targeted cell death. These include:

1) Cancer chemotherapy;
2) antibacterial treatments;
3) antifungal treatments;
4) the treatment of AIDS/HIV;
5) the treatment of multiple sclerosis; and

The agents may be used in a number of ways. For instance, systemic administration may be required in which case the agents may be contained within a composition, which may, for example, be ingested orally in the form of a tablet, capsule or liquid. Alternatively the agents may be administered by injection into the blood stream. Injections may be intravenous (bolus or infusion) or subcutaneous (bolus or infusion). The compounds may also be administered by inhalation (e.g. intranasally).

The agents may also be incorporated within a slow or delayed release device. Such devices may, for example, be inserted under the skin and the compound may be released over weeks or even months. The devices may be particularly advantageous when an agent is used which would normally require frequent administration (e.g. at least daily ingestion of a tablet or daily injection).

It is preferred that second agents according to the invention are initially dissolved in solvents such as DMSO before dilution in aqueous solution for the preparation of liquid medicaments.

The agents may be formulated as prodrugs. Such prodrugs may be stored as inactive and stable medicaments which are subsequently activated.

It will be appreciated that the amount of an agent required is determined by biological activity and bioavailability that in turn depends on the mode of administration and the physicochemical properties of the agents employed. The frequency of administration will also be influenced by the abovementioned factors and particularly the half-life of the agents within the subject being treated.

Known procedures, such as those conventionally employed by the pharmaceutical industry (e.g. *in vivo* experimentation, clinical trials etc), may be used to establish specific formulations of agents and precise therapeutic regimes (such as daily doses and the frequency of administration).

regime) or at 3 or 4 hourly intervals thereafter. Alternatively a slow release device may be used to provide optimal doses to a patient without the need to administer repeated doses. A preferred route of administration is by intravenous infusion. Administration may be over several hours or even days.

A preferred means of using protein or peptide agents is to deliver such agents to the target tissue by means of gene therapy. For instance, gene therapy may be used to decrease expression of Topo I or HSP90, decrease expression of enzyme(s) responsible for the intracellular synthesis of Topo I or HSP90, increase expression of a protein which promotes breakdown of Topo I or HSP90. Therefore according to a fourth aspect of the present invention there is provided a delivery system for use in a gene therapy technique, said delivery system comprising:

(i) a first DNA molecule encoding for a protein which directly or indirectly attenuates Topoisomerase I activity; and

(ii) a second DNA molecule encoding for a protein which directly or indirectly inhibits Heat Shock Protein 90 activity;

wherein said DNA molecules are capable of being transcribed to allow the expression of said proteins and thereby be effective for chemotherapy.

The delivery systems according to the fourth aspect of the invention are highly suitable for achieving sustained levels of a protein which are chemotherapeutically active over a longer period of time than is possible for most conventional therapeutic regimes. The delivery system may be used to induce continuous protein expression from cells in a target tissue that have been transformed with the DNA molecule. Therefore, even if the proteins have a very short half-life as agents *in vivo*, therapeutically effective amounts of the proteins may be continuously expressed from the treated tissue.

Furthermore, the delivery system of the invention may be used to provide the DNA molecules (and thereby the proteins which are active therapeutic agents) without the need to use conventional pharmaceutical vehicles such as those required in tablets, capsules or liquids.

The DNA molecules may (but not necessarily) be one which becomes incorporated in the DNA of cells of the subject being treated. Undifferentiated cells may be stably transformed leading to the production of genetically modified daughter cells (in which case regulation of expression in the subject may be required e.g. with specific transcription factors or gene activators). Alternatively, the delivery system may be designed to favour unstable or transient transformation of differentiated cells in the subject being treated. When this is the case, regulation of expression may be less important because expression of the DNA molecules will stop when the transformed cells die or stop expressing the proteins (ideally when chemotherapy is no longer required).

The delivery system may provide the DNA molecules to the subject without them being incorporated in a vector. For instance, the DNA molecules may be incorporated within liposomes or virus particles. Alternatively the "naked" DNA molecules may be inserted into a subject's cells by a suitable means e.g. direct endocytotic uptake.

The DNA molecules may be transferred to the cells of a subject to be treated by transfection, infection, microinjection, cell fusion, protoplast fusion or ballistic bombardment. For example, transfer may be by ballistic transfection with coated gold particles, liposomes containing the DNA molecules, viral vectors (e.g. adenovirus) and means of providing direct DNA uptake (e.g. endocytosis) by application of the DNA molecules directly to the target tissue topically or by injection.

The discovery that Topo I and HSP90 interact has enabled the inventor to develop a drug screening assay system for testing the efficacy of candidate drugs as chemotherapeutic agents. Therefore the two interacting proteins HSP90 and Topoisomerase I may be used as a complex target for new drug development in which both proteins are contemporaneously or sequentially targeted for new mammalian, fungal and anti bacterial agents.

are known to the art. Preferably a yeast two-hybrid interaction trap is employed. Yeast two-hybrid screening is a strategy for screening for interaction between proteins. Yeast two-hybrid screening used according to the invention may involve expression of translational fusions of (a) Topoisomerase I and part of a reporter gene; and (b) Heat Shock Protein 90 fused in-frame with the other part of the reporter gene. When the fusion proteins are expressed, interaction between (a) and (b) allows the reporter to assemble and generate a signal. Test compounds that represent candidate chemotherapeutic agents prevent interaction between (a) and (b) and may be identified because no reporter signal is produced from samples containing the candidate.

It will be appreciated that any other form of interaction trap may be used to put the invention into practice. Suitable examples included techniques such as mammalian two-hybrid, bacterial two-hybrid or alternatively various types of pull down assay.

When the methods relate to the disruption of protein-protein interactions based on the yeast two-hybrid technique it is preferred that yeast are used that are permeable to the tested compounds. Examples of drug permeable yeast which may be used according to the invention include MDS or ISE 2 mutations (e.g. strains carrying these mutations (ISE2), JJ700, BJ201). Suitable strains are disclosed in Hammonds *et al.* Antimicrob Agents Chemother. 1998 Apr;42(4):889-94.

It will be appreciated that the methods according to the fifth or sixth aspects of the invention may be adapted to identify compounds that promote interaction between Topoisomerase I and Heat Shock Protein 90 (rather than inhibit such interaction) Such an adapted test represents a good method for evaluating whether or not a test compound is likely to be carcinogenic. Therefore according to a seventh aspect of the present invention there is provided a method of screening a compound, to test whether or not said compound is carcinogenic, comprising exposing said compound to Topoisomerase I and Heat Shock Protein 90 to evaluate whether or not said compound promotes interaction between Topoisomerase I and Heat Shock Protein 90.

The method according to the eighth aspect of the invention may be adapted for determining the sensitivity of a subject to a specific combination of first and second agents according to the invention (i.e. an HSP90 inhibitor and a Topo I inhibitor). Thus according to a ninth aspect of the present invention there is provided an *in vitro* method for evaluating the suitability of chemotherapeutic treatment for administration to a subject, comprising:

(i) detecting the level of activity or expression levels of HSP90 and Topo I from a sample of cells from said subject; and

(ii) comparing the level of activity or expression levels of HSP90 and Topo I in said sample relative to activity expression levels of HSP90 and Topo I from a non-cancerous sample.

According to a tenth aspect of the present invention there is provided an *in vitro* method for monitoring the effectiveness of a chemotherapy for treating a subject, comprising:

(i) detecting the level of activity or expression levels of HSP90 and Topo I from a sample of cells from said subject; and

(ii) comparing the level of activity or expression levels of HSP90 and Topo I in said sample relative to activity expression levels of HSP90 and Topo I from a non-cancerous sample.

The invention will be further illustrated in the non-limiting Example and figures, in which:

Figure 1a illustrates the results of an immuno-precipitation assay with an HSP90 antibody in which protein from HCT116p53wt was subjected to a Western blot using Topo I as a probe;

Figure 1b illustrates the results of an immuno-precipitation assay with a Topo I antibody in which protein from HCT116p53wt was subjected to a Western blot using HSP90 as a probe;

Figure 2a illustrates the effect of Irenotecin alone on cell growth;

Figure 2b illustrates the effect of Geldanamycin alone on cell growth;

## EXAMPLE 1

Experiments were conducted that established HSP90 and Topoisomerase I interact and influence cell growth. This discovery lead the inventor to develop the various aspects of the invention described herein.

## METHODS

***Established cell culture***

The isogenic p53 human colon cancer cell line (WT and KO), HCT116 (see Hwang *et al.* Nat Med 2001 Nov 7(11):1255). Cells were maintained in McCoys 5A medium (Sigma) supplemented with 10 % foetal calf serum (Gibco) at 37°C in a 5 % $CO_2$ enriched humidified environment, Penicillin and Streptomycin.

Standard cell lines as above except:
K562 RMPI 1640 (Sigma), SK-MEL-3 McCoys (Sigma), OAW42 DMEM supplemented with 1mM sodium pyruvate 10μg/ml insulin & NCI-H125 RPMI 1640 (Sigma), HT29 DMEM (Sigma).

***Immunoprecipitations***

100 mm dishes were seeded with 3 x $10^6$ cells and allowed to adhere overnight. Media was placed with fresh media alone (control) or containing a Topo I inhibitor (e.g. Irinotecan) for 24 hours. Cells were washed twice with wash buffer (0.4 mM EDTA, 10 mM sodium fluoride, 10 mM sodium pyrophosphate, 0.4 mM sodium orthovanadate) and incubated on ice with 250 μl cell lysis buffer (50 mM Tris HCl pH 8.0, 425 mM NaCl, 1 mM EDTA, 10 mM sodium fluoride, 0.5 mM sodium orthovanadate, 1 % v/v igepal CA-630, 5 % w/v deoxycholic acid, 0.1 % w/v SDS) containing protease inhibitor cocktail III (Calbiochem). Cells were scraped on ice, sonicated for 30 seconds and cell debris removed by centrifugation at 14,000 x g for 30 minutes at 4°C. Cell lysates were then pre-cleared by incubation with 25 μl of 10 % w/v protein A sepharose CL-4B (Amersham Pharmacia Biotech) in PBS for 1 hour rotating at 4°C. Samples were spun briefly at maximum speed in a 4°C benchtop centrifuge and supernatants removed to fresh microfuge tubes. 5 μg of either anti-topoisomerase I or anti-heat shock protein 90β (Labvision) antibodies were added to cell lysates and incubated at 4°C overnight. 50 μl of 10 % w/v protein A sepharose in PBS was added and samples allowed to precipitate by rotating at 4°C for 1 hour. Samples were spun briefly at maximum speed in a 4°C benchtop centrifuge and supernatants discarded. Immunoprecipitates were washed with 250 μl cell lysis buffer, resuspended in 60 μl IPG buffer (7 M urea, 2 M thiourea, 4 % w/v CHAPS, 40 mM Tris base, 1% w/v DTT) and analysed by one-dimensional (1-D) electrophoresis.

***1-D electrophoresis and immunoblotting***

Total protein extracts and immunoprecipitations were separated by 7.5 % or 12 % SDS-PAGE under reducing conditions. Gels were then either stained using Colloidal blue concentrate (Sigma) in 20 % v/v methanol or blotted onto nitrocellulose membrane. Blots were probed with either rabbit primary antibodies against human

## EXAMPLE 2

Example 1 illustrates there was a physical interaction between Topo I and HSP90. The inventor therefore tested the effect of combining drugs that had a specific effect on Topo I and a specific effect on HSP90. The combination of an HSP90 inhibitor and a topoisomerase I inhibitor show a synergistic effect (see below).

### METHODS

*Growth inhibition assay*
96 well flat-bottomed plates were seeded with 3 x $10^3$ cells per well and allowed to adhere overnight. Media was then replaced with fresh media alone (control) or containing test drugs eg 0 – 100μM Irinoitecan, 50 to 200 nM geldanamycin (GA) and combinations of both. At fixed time points, cells were fixed with 3:1 methanol:acetic acid and stained with 0.4 % w/v sulforhodamine B (Sigma) in 1 % v/v acetic acid for 30 minutes. Plates were then washed twice with 1 % v/v acetic acid, the dye solubilised with 100 μl per well of 10 mM Tris pH 10.4 and read at A570nm using a Benchmark microplate reader (BioRad).

*Clonogenic assay*
Cells were plated at a density of 1000 cells per well in 6 well plates and allowed to adhere overnight. Cells were treated with e.g. 0.5 to 50 μM VP16, 50 to 1500 nM GA or combinations of the two for 1 hour. Cells were then washed twice with PBS and re-incubated with fresh medium for 10 days. Media was then removed and cells were fixed with 70 % v/v methanol for 1 minute. Cells were then stained with 0.2 % w/v crystal violet in 70 % v/v ethanol for 10 seconds, washed with $dH_2O$ and allowed to air dry. The number of colonies formed of > 50 cells each were counted.

Drugs were used in the following concentrations for growth inhibition and clonagenic assays:
Geldanamycin 1-1500 nM
Irinotecan 0.01-100μM

*Flow cytometry protocol for cell cycle analysis.*

1. Seed cells eg HCT116 +/+ or K562 cells in small petri dish or 6 well plate using 5ml of 1 x $10^6$ cells/ml in appropriate medium. For HCT116+/+ cell line use McCoys 5A Medium supplemented with 10% Foetal Calf Serum (FCS) and Penicillin and Streptomycin. For K562 cell line use RPMI 1640 Medium supplemented with 10% FCS and Penicillin and Streptomycin.
2. Leave to attach overnight for adherent cell lines in incubator at 37$^{o}$C 5% $CO_2$ atmosphere.*

## CLAIMS

1. A use of a first agent that attenuates Topoisomerase I (Topo I) activity and a second agent that inhibits Heat Shock Protein 90 (HSP90) activity in the manufacture of a medicament for contemporaneous or sequential administration in chemotherapy.

2. The use according to claim 1 wherein the first agent is a compound selected from:
   (i) compounds that bind to Topo I and inhibit its activity;
   (ii) compounds which prevent the transcription, translation or expression of Topo I;
   (iii) compounds which inhibit release of Topo I from intracellular stores; and
   (iv) compounds which increase the rate of degradation of Topo I.

3. The use according to claim 1 or 2 wherein the first agent is a cleavable-complex inhibitor.

4. The use according to claim 1 or 2 wherein the first agent is Camptothecin or a derivative or analogue thereof.

5. The use according to claim 1 or 2 wherein the first agent is Topotecan or a derivative or analogue thereof.

6. The use according to claim 1 or 2 wherein the first agent is Irinotecan or a derivative or analogue thereof.

7. The use according to claim 1 or 2 wherein the first agent is Camptostar (CPT-11) or a derivative or analogue thereof.

8. The use according to any preceding claim wherein the second agent is a compound selected from:

antifungal treatments;

the treatment of AIDS/HIV;

the treatment of multiple sclerosis; or

the killing and inhibition of proliferation of any organism.

18. The use according to any preceding claim wherein the chemotherapy is for prophylactic treatment.

19. A delivery system for use in a gene therapy technique, said delivery system comprising:

(i) a first DNA molecule encoding for a protein which directly or indirectly attenuates Topoisomerase I activity; and

(ii) a second DNA molecule encoding for a protein which directly or indirectly inhibits Heat Shock Protein 90 activity;

wherein said DNA molecules are capable of being transcribed to allow the expression of said proteins and thereby be effective for chemotherapy.

20. The use of a delivery system according to claim 19 for the manufacture of a medicament for use in chemotherapy.

21. The use according to claim 20 for the treatment of conditions defined by any one of claims 11 to 18.

22. A method of screening a first and a second compound, to test whether or not said compounds has efficacy for use in combination as a chemotherapy, comprising:

(a) exposing said compounds to Topoisomerase I and evaluating whether or not said compounds bind thereto;

(b) exposing said compounds to Heatshock Protein 90 and evaluating whether or not said compounds bind thereto; and

(c) selecting a first and second compound, wherein at least one compound binds to Topoisomerase I and at least one compound binds to Heatshock Protein 90 for use in combination as a chemotherapy.

(i) detecting the level of activity or expression levels of Heat Shock Protein 90 and Topoisomerase I from a sample of cells from said subject; and

(ii) comparing the level of activity or expression levels of Heat Shock Protein 90 and Topoisomerase I in said sample relative to activity expression levels of Heat Shock Protein 90 and Topoisomerase I from a non-cancerous sample.

30. An *in vitro* method for monitoring the effectiveness of a chemotherapy for treating a subject, comprising:

(i) detecting the level of activity or expression levels of Heat Shock Protein 90 and Topoisomerase I from a sample of cells from said subject; and

(ii) comparing the level of activity or expression levels of Heat Shock Protein 90 and Topoisomerase I in said sample relative to activity expression levels of Heat Shock Protein 90 and Topoisomerase I from a non-cancerous sample.

Fig 1a

**Immuno-precipitation Assay: HCT116 p53wt cells (treated for 24 hrs) Western Blot probed with Topo-1**

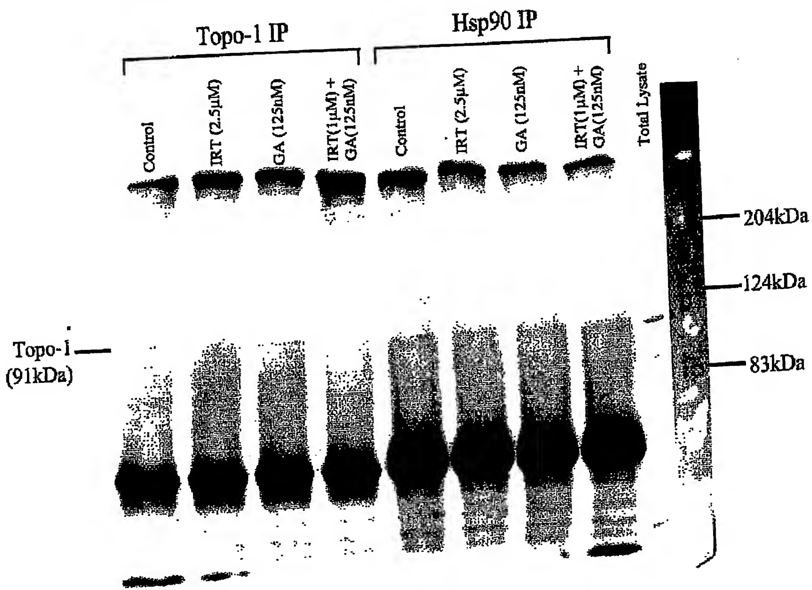


Fig 1b

**Immuno-precipitation Assay: HCT116 p53wt cells (treated for 24 hrs) Western Blot probed with Hsp90**

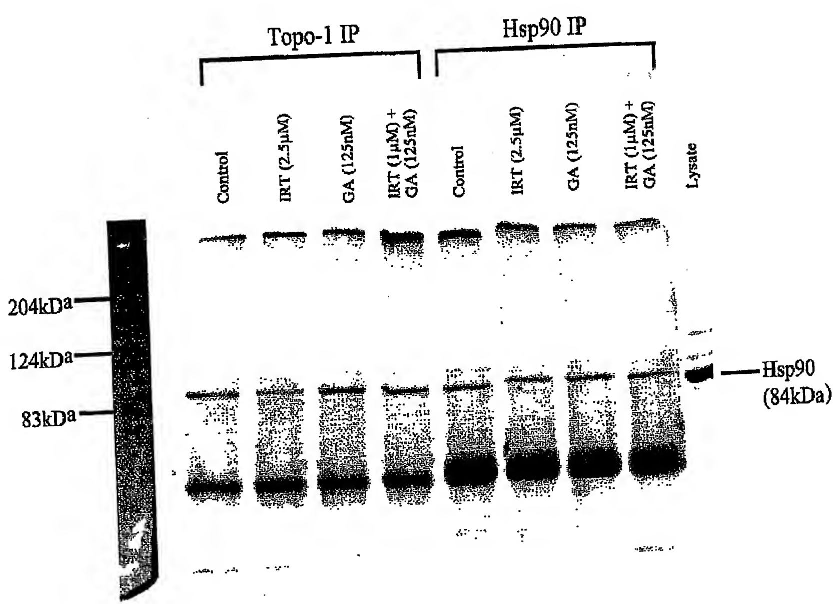
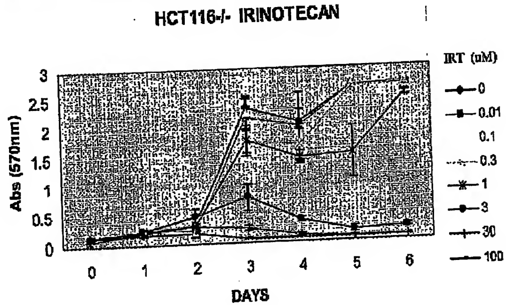


Fig 2a

## GROWTH INHIBITION CURVES IRINOTECAN ONLY



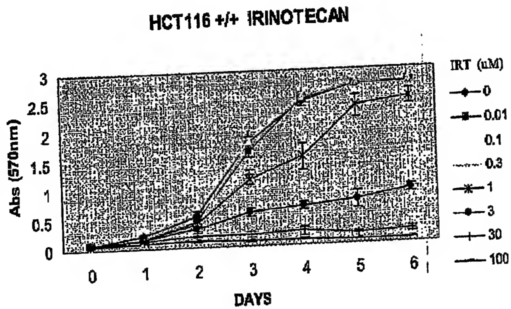
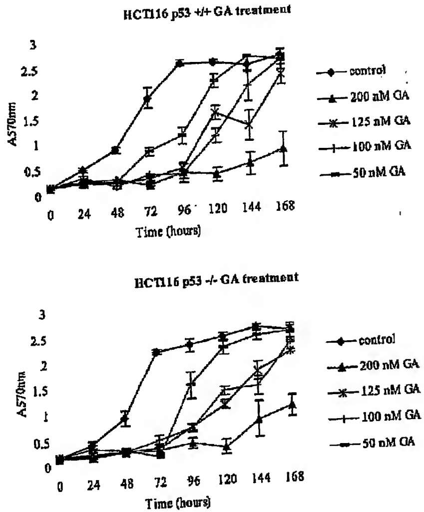



Fig2b

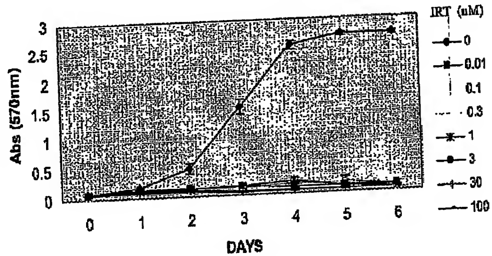
# GROWTH INHIBITION CURVES GELDANAMYCIN ONLY



# GROWTH INHIBITION CURVES FOR COMBINATION DRUG TREATMENT

Fig2c

HCT116 +/+ IRINOTECAN + GA(125nm)




HCT116 -/- IRINOTECAN + GA(125nm)

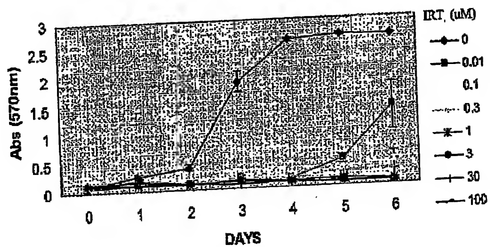
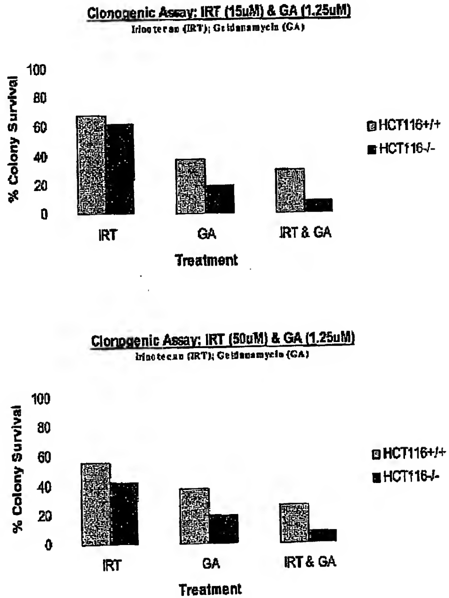


Fig 3


Clonogenic Assay: IRT (15uM) & GA (1.25uM)
Irinotecan (IRT); Geldanamycin (GA)
% Colony Survival
100
80
60
40
20
0
IRT
GA
IRT & GA
Treatment
HCT116+/+
HCT116-/-
Clonogenic Assay: IRT (50uM) & GA (1.25uM)
Irinotecan (IRT); Geldanamycin (GA)
% Colony Survival
100
80
60
40
20
0
IRT
GA
IRT & GA
Treatment
HCT116+/+
HCT116-/-